

SUPPORTING INFORMATION

X-ray Structure Analysis of Indazolium *trans*-[Tetrachlorobis(1H-indazole)ruthenate(III)] (KP1019) Bound to Human Serum Albumin Reveals two Ruthenium Binding Sites and Provides Insights into the Drug Binding Mechanism

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1. Data Collection and Processing

X-ray diffraction data were collected under cryo-conditions (100 K) at beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France using a PILATUS 6M detector. The wavelength of the beam was 0.973 Å. Diffraction data of the HSA-Myr-KP1019 complex were collected at 3.2 Å resolution (oscillation range: 0.15°, exposure time: 0.08 s, crystal to detector distance: 387 mm). The data were processed with XDS¹ and the structure solved with programs from the CCP4² and PHENIX³ suites. Data collection and refinement statistics are summarized in Table S1.

2. Structural Analysis and Refinement

The crystal belonged to the C 1 2 1 space group, the most common space group for HSA structures according to the PDB. The unit-cell parameters were $a = 181.11$ Å, $b = 38.06$ Å, $c = 94.95$ Å, $\alpha = 90.00^\circ$, $\beta = 105.06^\circ$ and $\gamma = 90.00^\circ$. The asymmetric unit contained one HSA molecule and the solvent content was 48.8 % with a Matthew's coefficient of 2.40 Å³/Da (calculated by PHENIX Xtriage). The structure of HSA-Myr-3'-azido-3'-deoxythymidine-salicylic acid (PDB code 3B9M)⁴ was used as search model for the molecular replacement procedure. Crystallographic refinement was carried out with phenix.refine (PHENIX) and manual rebuilding with Coot.⁵ For the final refinement steps ruthenium ions including coordinating solvent molecules (incorporated as water molecules) were inserted into the electron density map and a restraint file was created restraining the geometry of the ruthenium coordination sphere (limits on the Ru-N(His) distances were set to 2.1 ± 0.3 Å, whereas Ru-OH₂ distances were limited to 2.5 ± 0.8 Å). The occupancies and B-factors of the incorporated ligands were separately refined. Furthermore, six Myr molecules were incorporated into the model despite the fact that only slight electron density was found for each Myr, however, the fatty acid chains became clearly visible when contouring the map to 0.5 . Refinement statistics are summarized in Table S1. The final X-ray structure was deposited to the Protein Data Bank (PDB) with the entry ID 5IFO.

3. Crystallographic Data Collection and Refinement Statistics

Table S1. Data collection and refinement statistics.

Crystal data

Space group	<i>C</i> 1 2 1
<i>a</i> , <i>b</i> , <i>c</i> (Å)	181.11, 38.06, 94.95
α, β, γ (°)	90.00, 105.06, 90.00
Molecules per asymmetric unit	1
Matthews coefficient (Å ³ Da ⁻¹)	2.40
Solvent content (%)	48.8
Max. Resolution (Å)	3.2

Data collection and processing

Wavelength (Å)	0.973
Resolution limits (Å)	45.84 - 3.2 (3.314 - 3.2)
No. of observed reflections	35381 (3465)
No. of unique reflections	10508 (1006)
Redundancy	3.4
$R_{p.i.m.}^{[a]}$	0.041 (0.127)
$R_{merge}^{[b]}$	0.065 (0.203)
CC _{1/2}	0.998 (0.983)
CC*	1 (0.99)
Completeness (%)	98 (98)
$\langle I / I \rangle$	17.89 (5.36)

Refinement statistics

Resolution (Å)	3.2
Reflections used	10501 (1003)
$R_{work}^{[e]}$ (%)	24.48 (35.57)
$R_{free}^{[f]}$ (%)	26.20 (36.09)
Average B-factor (Å ²)	80.15

Ramachandran plot^[g]

Most favoured regions (%)	96
Additional allowed regions (%)	4
Disallowed regions (%)	0.30
PDB code	5IFO

Statistics for the highest resolution shell are shown in parantheses. [a] $R_{p.i.m.} = \frac{1}{N(hkl)} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_i I_i(hkl)$, where $I_i(hkl)$ is the *i*th observation of reflection *hkl* and $\langle I(hkl) \rangle$ is the weighted average intensity for all observations of reflection *hkl*. [b] $R_{merge} = \frac{1}{N(hkl)} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_i I_i(hkl)$. [c] Mean anomalous difference in units of its estimated standard deviation $|F(+)-F(-)|/ \sigma$, $F(+)$ and $F(-)$ are structure factors obtained from the merged intensity observations in each parity class. [d] Percentage of correlation between random half-sets of anomalous intensity differences. [e] $R_{work} = \frac{|F_{calc}| - |F_{obs}|}{|F_{obs}|} \cdot 100$, where F_{calc} and F_{obs} are the calculated and observed structure factor amplitudes, respectively. [f] R_{free} is calculated for randomly chosen 5 % of the reflections for each dataset. [g] Calculated using COOT validation.

4. Chemical Structure of NAMI-A and AziRu

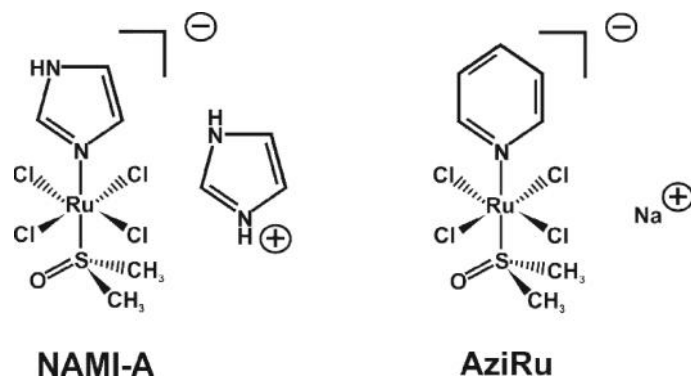


Figure S1. Chemical structures of imidazolium *trans*-[tetrachlorobis(1H-imidazole)(S-dimethylsulfoxide)ruthenate(III)] (NAMI-A) and sodium *trans*-[tetrachlorobis(1H-pyridine)(S-dimethylsulfoxide)ruthenate(III)] (AziRu).

5. References

- (1) Kabsch, W. *XDS. Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 125–132.
- (2) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 Suite and Current Developments. *Acta Crystallogr. D Biol. Crystallogr.* **2011**, *67*, 235–242.
- (3) Adams, P. D.; Afonine, P. V.; Bunkóczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L.-W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. *PHENIX: A Comprehensive Python-Based System for Macromolecular Structure Solution. Acta Crystallogr. D Biol. Crystallogr.* **2010**, *66*, 213–221.
- (4) Zhu, L.; Yang, F.; Chen, L.; Meehan, E. J.; Huang, M. A New Drug Binding Subsite on Human Serum Albumin and Drug–drug Interaction Studied by X-Ray Crystallography. *J. Struct. Biol.* **2008**, *162*, 40–49.
- (5) Emsley, P.; Cowtan, K. *Coot: Model-Building Tools for Molecular Graphics. Acta Crystallogr. D Biol. Crystallogr.* **2004**, *60*, 2126–2132.